

INFLUENCE OF TEMPERATURE ON THE ASPHYXIATION OF YOUNG GOLDFISH (*CARASSIUS AURATUS* L.) UNDER VARIOUS TENSIONS OF OXYGEN AND CARBON DIOXIDE

F. E. J. FRY, V. S. BLACK, AND EDGAR C. BLACK

*Ontario Fisheries Research Laboratory, University of Toronto, Canada and Department of
Physiology, Dalhousie University, Halifax, Canada*

INTRODUCTION

In their preliminary work on the sensitivity of Ontario fishes to carbon dioxide as measured by the asphyxial tension of oxygen of fish in sealed jars containing various concentrations of dissolved carbon dioxide, Fry and Black (1938) observed a difference in the response of two sample groups of the minnow, *Chrosomus eos*. The group taken in the autumn from a cold stream near Toronto was found to be appreciably more sensitive than the group from a bog lake in Algonquin Park at the height of the summer warming. These data were subsequently published in a review (Fry, 1939). At that time there was no indication as to the cause of this difference since the two groups came not only from different parts of the country but also from different habitats.

Further measurements were made in Algonquin Park in 1939 when the work was continued into early fall. Some comparisons were made of curves showing sensitivity to carbon dioxide for the same species from the same locality at different times of year. The same effect was noted as for *Chrosomus eos*, although the data were rather scanty. Observations of the same sort were made again in 1940 and, in particular, material was gathered for the brown bullhead (*Ameiurus nebulosus*). The results are illustrated in Figure 1. There is an increased sensitivity in late summer or early fall when the water is cooling. These tests were carried out in water of the same temperature as that in which the animals were captured.

Reasoning by analogy from the fact that cold water species such as the trout fail to take up oxygen in the presence of much lower tensions of carbon dioxide than do warm water species such as the brown bullhead, it was concluded that probably there would be some such trend within each species and that concomitant with the process of thermal adaptation there might be a change in respiratory sensitivity in the direction observed.

Meanwhile an opportunity to test this experimentally had presented itself in February 1939, when two of the authors in collaboration with Professor Laurence Irving and using the facilities of the experimental hatchery at Cornell University, measured the respiratory sensitivity of the speckled trout at 1° C, the temperature of the hatchery water at that time. The respiratory sensitivity was also measured of a group of these fish which had spent 24 hours at 13° C. The sensitivity of the group subjected to the warm water for even that short period of time was appreciably decreased.

This experiment, however, was only of a preliminary nature and at that time it was not possible to carry it to its logical conclusion. For this reason we undertook to do a further series of experiments to determine the effect of temperature on the respiratory sensitivity of thermally adapted goldfish. The temperatures chosen covered the biokinetic range of temperature for this species. Goldfish were maintained at each temperature level for a period of time quite sufficient to ensure a thorough thermal adaptation. The goldfish was chosen because of its suitability as a laboratory animal, because the thermal tolerance for the young goldfish had been

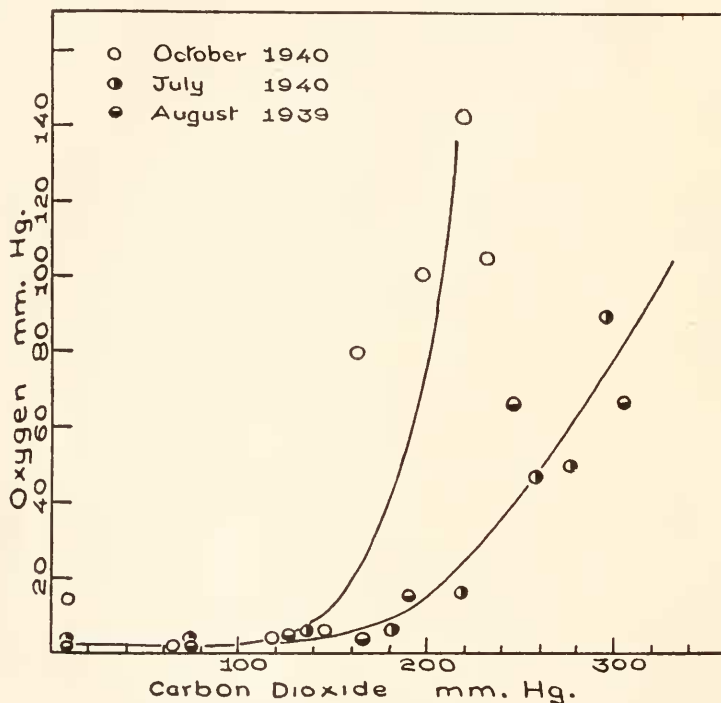


FIGURE 1. Seasonal variation in the extent to which the brown bullhead can reduce the oxygen content of water in sealed bottles in the presence of various tensions of carbon dioxide. In July and August the fish were acclimated to approximately 20° C., in October to 13° C. They were tested at the acclimation temperatures in each instance.

plotted with some exactitude (Fry *et al.*, 1942), and also because the rate of thermal adaptation of goldfish was being measured (Brett, 1946) at the time these experiments were begun.

MATERIAL AND METHODS

The goldfish, *Carassius auratus* (Linnaeus), were the ordinary long finned commercial variety of goldfish purchased from commercial dealers in Ontario. They were approximately one year old, of both sexes, and varied from 2½ to 4 inches in length. The fish were fed fox chow during the period of acclimation.

A group of fish was acclimatized to one of the following temperatures: 1° C., 7° C., 15° C., 20° C., 25° C., and 32° C. The overall allowance for acclimation of each

group of fish from 7° to 32° was 1° C. or less per day until the desired temperature was reached. Since complete acclimation to low temperatures takes a longer time (Doudoroff, 1942) the fish used for the 1° C. curve were put from a 7° C. tank into a 1° C. tank and left for two months.

When acclimation was complete each fish was put in a bottle of water (275 ml. capacity) of the same temperature and containing a known tension of carbon dioxide and at least 150 mm. tension of oxygen. During the experiment the sealed bottles containing the fish were kept in a water bath at the temperature of acclimation. When all respiratory movements of the fish had ceased the water in the bottle was analyzed for free carbon dioxide and oxygen. The tension of carbon dioxide was determined as follows: a small bubble of air (0.1–0.3 ml.) was equilibrated with 35 ml. of the water for five or more minutes at the temperature of the experiment at room pressure; the bubble was then transferred to a micro-gas-analyzer (after Krogh, 1908) and the carbon dioxide content of the gas phase was found by absorption of carbon dioxide with $\frac{1}{4}$ N potassium hydroxide. The tension was obtained by multiplying the fraction of carbon dioxide in the gas phase by the barometric pressure minus the vapor pressure of the water at the temperature of the experiment.

Dissolved oxygen was determined by the unmodified Winkler method, using a 50 ml. sample of water. The pressure of oxygen was calculated by relating the quantity of oxygen found to the solubility at the temperature of the experiment and multiplying the fraction obtained by 760.

RESULTS

The data for the experiments at each of the six temperatures (1° C., 7° C., 15° C., 20° C., 25° C., and 32° C.) are given in Table I and illustrated in Figure 2. To

TABLE I

The extent to which goldfish acclimated to various temperatures can reduce the oxygen content of water containing dissolved carbon dioxide. Each pair of values is the mean of three to five determinations

1° C.		7° C.		15° C.		20° C.		25° C.		32° C.	
CO ₂ mm. Hg	O ₂ mm. Hg	CO ₂ mm. Hg	O ₂ mm. Hg	CO ₂ mm. Hg	O ₂ mm. Hg	CO ₂ mm. Hg	O ₂ mm. Hg	CO ₂ mm. Hg	O ₂ mm. Hg	CO ₂ mm. Hg	O ₂ mm. Hg
20	5.5	27	3.5	17	2.9	34	7.5	25	4.7	14	8.0
64	4.9	79	3.5	66	3.5	88	8.3	141	13	144	8.0
87	48	100	5.8	110	5.9	120	2.9	172	7.6	168	7.0
98	24	113	16	131	12	139	7.3	200	42	186	7.3
111	46	128	38	144	49	154	41	224	120	212	46
141	66	138	66	153	49	166	25	286	272	226	102
				171	74	197	80			240	216
				192	75	242	139				
				237	163						

reduce the spread the original figures have been grouped into averages of 3 to 5 determinations. This averaging was carried out by arranging all the values in a particular series in order of the magnitude of the carbon dioxide tension and then averaging the values for consecutive groups.

The course at each temperature appears to be as follows. Up to a certain tension of carbon dioxide the oxygen in the bottle is reduced to a uniform and low level before death of the fish ensues. Above a certain carbon dioxide tension the level of the carbon dioxide influences the level to which the oxygen can be reduced, i.e., the greater the carbon dioxide content the higher the level of oxygen in the water at

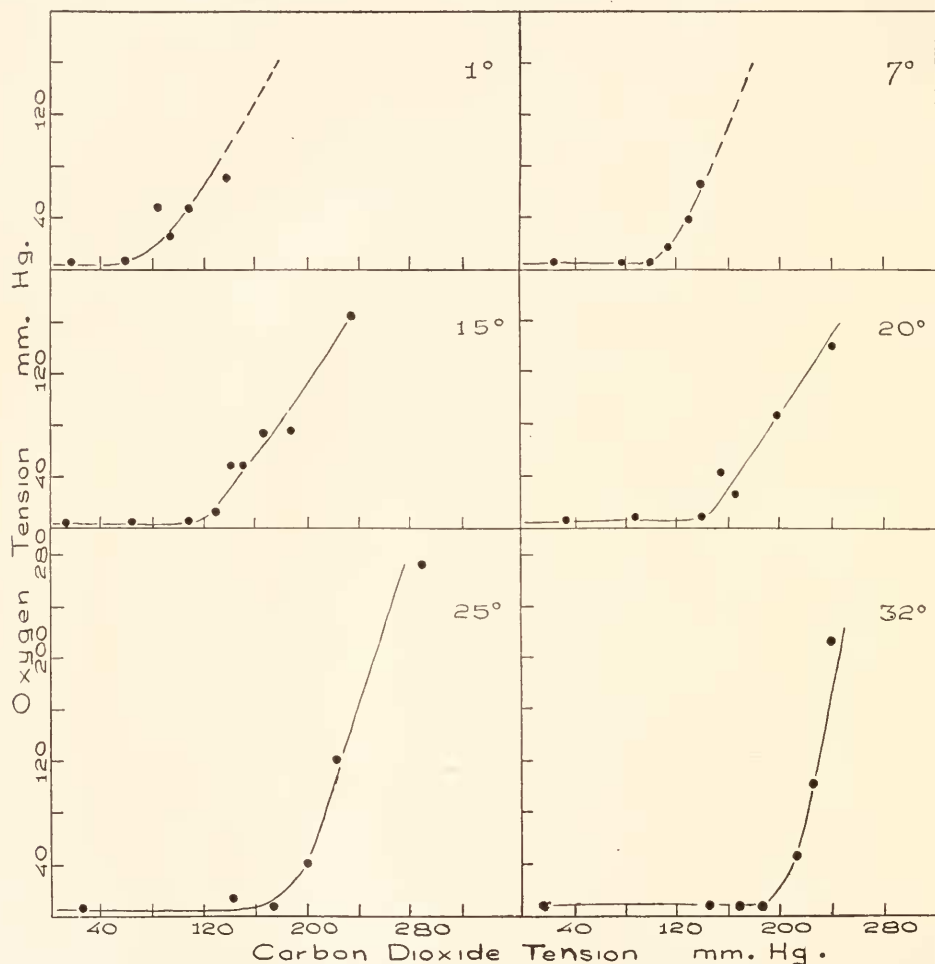


FIGURE 2. The extent to which goldfish can reduce the oxygen content of water in sealed bottles in the presence of dissolved carbon dioxide at various temperatures of acclimation. Each point is the average of three to five determinations.

asphyxiation. As the acclimation temperature is increased the level of carbon dioxide required to limit the level to which the oxygen is used also increases. There is some indication that the rising portion of the curve becomes steeper at the higher acclimation temperatures.

The data from the six curves in Figure 2 are brought together in Figure 3 where the change in sensitivity, i.e., the number of arbitrary units contained within

each curve, is plotted against the temperature of acclimation. The sensitivity of the fish seems to decrease almost directly with the increase in temperature of acclimation.



FIGURE 3. The relation between utilization of oxygen in the presence of dissolved carbon dioxide and the temperature to which goldfish are acclimated. The units of resistance to carbon dioxide given are the areas composed by oxygen \times carbon dioxide (as mm. Hg) between the resistance curves in Figure 2 and a horizontal line drawn across at 160 mm. Hg oxygen (air saturation). Sensitivity to CO_2 referred to in the text is a reciprocal function of resistance.

DISCUSSION

The ability of the fish to use oxygen in the presence of carbon dioxide as measured here, varies considerably in goldfish adapted to various temperatures within their biokinetic range. The difference between goldfish acclimated to water of 1°C . and those acclimated to water of 32°C . is of the same order as the differences between the common sucker (*Catostomus commersonnii*) and the brown bullhead

(*Ameiurus nebulosus*) when these species are acclimated to about 20° C. (Fry, 1939).

The change in the respiratory sensitivity attendant on change in the environmental temperature is such that when goldfish are adapted to low temperatures their respiratory sensitivity tends toward the type found in cold water species (common sucker): when adapted to high temperatures the respiratory sensitivity resembles that of warm water species (brown bullhead).

Other changes in the respiratory metabolism of fish have been found to be attendant on changes in thermal adaptation (Wells, 1935; Sumner and Wells, 1935; Sumner and Lanham, 1942; Sumner and Doudoroff, 1938). Indeed Sumner and Doudoroff used the change in resistance time to cyanide poisoning as an index of change in thermal adaptation. The change shown in our work appears however to be unique in that the absolute level of respiratory sensitivity is lower at a higher temperature.

While no definite conclusions can be drawn it is interesting to speculate on the change of organization which could bring about this change in respiratory sensitivity. Two phases of the circulatory systems of fish have been shown to be correlated with their respiratory sensitivity. Black (1940) noted that there were significant differences between four species of freshwater fish and the effect of carbon dioxide on the affinity of hemoglobin in whole blood for oxygen, and further, that the fish with the greater sensitivity to carbon dioxide are found in colder habitats. Hart (1945) showed a similar correlation between the stroke output of the heart and the respiratory sensitivity of a series of species. He also pointed out that the influence of the mechanical factor in respiratory transport may at times be so great as to displace the order of respiratory sensitivity of species which might be expected from a comparison of the chemical characteristics of their oxygen transport system. In addition to Hart's material another probable instance of this is found in the marine fish, the tautog (*Tautoga onitis*) and the toadfish (*Opsanus tau*). The respiratory tolerance of these fish is quite different, the toadfish being able to withstand up to 250 mm. of carbon dioxide whereas the tautog can only tolerate 150 mm. (Safford, 1940). The chemical properties of the blood of these fish for transporting oxygen in the presence of carbon dioxide are, however, very similar (Root, Irving, and Black, 1939).

Thus the change in respiratory sensitivity with change in temperature as displayed by goldfish adapted to their thermal environment may be a reflection of changes in either the mechanical or chemical aspects of their circulatory system and the problem remains to see to what extent each may be acting.

There is another change in the organism that may influence the measure of respiratory sensitivity as determined here for the goldfish. The areas enclosed above and to the left of the carbon dioxide-oxygen curves in Figures 1 and 2 are not entirely areas of tolerance in the sense that the animal could remain alive indefinitely under conditions represented by any combination of oxygen and carbon dioxide within these areas. At points in the marginal area near the limiting curve the animal is in a dying condition and its ability to transport oxygen will not depend on its tolerance towards those conditions but upon the length of time its organization can resist the ultimate breakdown. During the part of this period when the ventilatory and circulatory systems are still functioning, sub-minimal amounts of oxygen will be transported to the tissues of the dying animal. While these amounts of oxygen may be sub-minimal from the point of view of maintaining the life of the organism they

may be quite significant in extending the carbon dioxide-oxygen curve considerably beyond the region of tolerance. This effect was well shown by Wiebe *et al.* (1934) who demonstrated that after a fish introduced into a sealed container had extracted oxygen in the presence of a certain pH until it had died, a second subject introduced into the same container could reduce the oxygen still further by a significant amount before it also died.

Thus the decrease in the respiratory sensitivity at higher temperatures as measured by asphyxiation in a sealed bottle could in part be accounted for by increase in resistance of the tissues to oxygen lack or carbon dioxide excess, taking resistance to mean the length of time that an organism can resist a lethal condition before it finally succumbs. However one would not expect this factor to account for anything like the whole of the change in respiratory sensitivity observed.

When conditions are standard with respect to heredity and environment the respiratory sensitivity curves appear to offer a valid physiological yardstick whatever question there may be of exactly what these experiments measure. In the present series of experiments for instance the 20° curve was obtained in 1945 using material of the same age and purchased from the same source as that which was used for the determinations at the other temperatures in 1943, and as will be seen the data are quite consistent.

Necessity for complete acclimation to secure standard results becomes apparent when temperature effect over the biokinetic range at one level of thermal adaptation is considered. We have made two comparisons on fish acclimated to 20° C. In one instance the experiments were conducted in water at 12° C., in the other at 28° C. These data are given in Table II and may be compared with the values given for

TABLE II

The extent to which goldfish acclimated to 20° C. can reduce the oxygen content of water containing dissolved carbon dioxide at 12° C. and at 28° C. Each pair of values is the mean of four determinations

Acclimated to 20° Experiments at 12°		Acclimated to 20° Experiments at 28°	
CO ₂ mm. Hg	O ₂ mm. Hg	CO ₂ mm. Hg	O ₂ mm. Hg
24	5.7	28	7.9
82	5.4	69	7.4
100	8.6	121	11
111	9.8	140	18
127	19	157	60
152	32	169	25
181	100	186	141
.		217	184

20° C. in Table I. In both cases the respiratory sensitivity proved to be greater than that measured at the temperature of acclimation. The degree of difference that these results show is somewhat surprising in view of the fact that the difference in temperature at which the experiments were carried out was only 8 degrees in either direction from the acclimation temperature, while at the acclimation level chosen the lower lethal temperature is about 18° C. below and the upper lethal 15° C. above the acclimation temperature.

SUMMARY

1. By asphyxiating goldfish in sealed bottles of water containing dissolved carbon dioxide and oxygen, it was found that the ability of the fish to use oxygen in the presence of carbon dioxide increases as the acclimation temperature of the fish is raised.

2. Goldfish acclimated at 1° C. are limited in their ability to use oxygen when a tension of 60 mm. or more carbon dioxide is present in the water, whereas goldfish acclimated at 32° C. are not limited in the utilization of oxygen unless the carbon dioxide tension is 200 mm. or more. Fish at intermediate temperatures of 7°, 15°, 20° and 25° C. begin to show a decrease in the ability to use oxygen when they are in the presence of carbon dioxide tensions of 100, 120, 140, and 170 mm. respectively.

3. Reliable results could be obtained only after complete acclimation of the fish to the temperature at which the experiments were made. However, with acclimated fish of similar stock the result could be reproduced from year to year at the same season.

4. The explanation for this change in sensitivity to carbon dioxide in acclimated fish lies possibly in the effects of temperature on both the mechanical transport of the blood and the chemical transport of oxygen by the blood.

LITERATURE CITED

- BLACK, EDGAR C., 1940. The transport of oxygen by the blood of freshwater fish. *Biol. Bull.*, 79: 215-229.
- BRETT, J. R., 1946. Rate of gain of heat-tolerance in goldfish (*Carassius auratus*). *Univ. Toronto Stud. Biol. Series No. 53. Publ. Ont. Fish. Res. Lab.* 64: 1-28.
- DOUDOROFF, P., 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). *Biol. Bull.*, 83: 219-244.
- FRY, F. E. J., 1939. The position of fish and other higher animals in the economy of lakes. *Am. Assoc. for the Adv. of Sci., Publ. No. 10*: 132-142.
- FRY, F. E. J., AND E. C. BLACK, 1938. The influence of carbon dioxide on the utilization of oxygen by certain species of fish in Algonquin Park, Ontario. *Anat. Rec.*, 72: Supplement, 47.
- FRY, F. E. J., J. R. BRETT, AND G. H. CLAWSON, 1942. Lethal limits of temperature for young goldfish. *Rev. Can. de Biol.*, 1: 50-56.
- HART, J. S., 1945. The circulation and respiratory tolerance of some Florida fresh-water fishes. *Proc. Fla. Acad. Sci.*, 7: 221-246.
- KROGH, A., 1908. On micro-analysis of gases. *Skand. Arch. f. Physiol.*, 20: 279-288.
- ROOT, R. W., LAURENCE IRVING, AND E. C. BLACK, 1939. The effect of hemolysis upon the combination of oxygen with the blood of some marine fishes. *Jour. Cell. Comp. Physiol.*, 13: 303-313.
- SAFFORD, VIRGINIA, 1940. Asphyxiation of marine fish with and without CO₂ and its effect on the gas content of the swim bladder. *Jour. Cell. Comp. Physiol.*, 16: 165-173.
- SUMNER, F. B., AND P. DOUDOROFF, 1938. Some experiments upon temperature acclimatization and respiratory metabolism in fishes. *Biol. Bull.*, 74: 403-429.
- SUMNER, F. B., AND U. N. LANHAM, 1942. Studies of the respiratory metabolism of warm and cool spring fishes. *Biol. Bull.*, 82: 313-327.
- SUMNER, F. B., AND N. A. WELLS, 1935. Some relations between respiratory metabolism in fishes and susceptibility to certain anaesthetics and lethal agents. *Biol. Bull.*, 69: 368-378.
- WELLS, NELSON A., 1935. The influence of temperature upon the respiratory metabolism of the Pacific killifish, *Fundulus parvipinnis*. *Physiol. Zool.*, 8: 196-227.
- WIEBE, A. H., A. M. MCGAVOCK, A. C. FULLER, AND H. C. MARKUS, 1934. The ability of freshwater fish to extract oxygen at different hydrogen ion concentrations. *Physiol. Zool.*, 7: 435-448.